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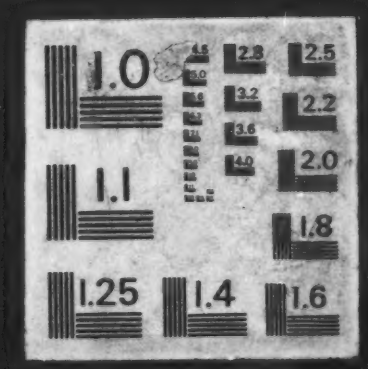

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Application of the Hall Detector and a Surface-Bonded Carbowax 20M Column to Analysis of Organochlorine Pesticides in Human Biological Samples

(U.S.) Health Effects Research Lab., Research Triangle Park, NC

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16. ABSTRACT

An evaluation of a gas chromatographic electrolytic conductivity detector system (Hall detector) was conducted for the determination and confirmation of selected organochlorine pesticides at sub-ppm concentration levels in human biological extracts. The sample extracts were also analyzed with electron capture gas chromatography for comparison purposes. The linear regression lines for both sets of data were calculated by the least squares method. A high degree of correlation between the data sets was found. Also evaluated as part of the electrolytic conductivity detector system was a column with Carbowax 20M bonded to the surface of the support. The effect of repeated injections of extracts with a high content of lipids on the performance of the Hall detector and column was investigated. Gel-permeation chromatography was found to be valuable as a technique for additional clean-up of certain extracts having an adverse effect on the performance of the Hall detector due to excessive lipid material.

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APPLICATION OF THE HALL DETECTOR AND A SURFACE-BONDED CARBOWAX 20M COLUMN TO ANALYSIS OF ORGANOCHLORINE PESTICIDES IN HUMAN BIOLOGICAL SAMPLES

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SUMMARY

An evaluation of a gas chromatographic electrolytic conductivity detector system (Hall detector) was conducted for the determination and confirmation of selected organochlorine pesticides at sub-ppm concentration levels in human biological extracts. The sample extracts were also analyzed with electron capture gas chromatography for comparison purposes. The linear regression lines for both sets of data were calculated by the least squares method. A high degree of correlation between the data sets was found. Also evaluated as part of the electrolytic conductivity detector system was a column with Carbowax 20M bonded to the surface of the support. The effect of repeated injections of extracts with a high content of lipids on the performance of the Hall detector and column was investigated. Gel-permeation chromatography was found to be valuable as a technique for additional clean-up of certain extracts having an adverse effect on the performance of the Hall detector due to excessive lipid material.

INTRODUCTION

Coulson[1] first reported on the use of an electrolytic conductivity detector for the determination of organochlorine compounds eluting from a gas chromatograph. Improved modifications[2,3] of the Coulson detector followed.

Hall[4] recently designed a micro-electrolytic conductivity detector cell that reportedly possessed a significant increase in sensitivity over the Coulson system. In addition, this system is simpler in design employing a Teflon® and stainless-steel conductivity cell. The improved sensitivity is not only due to the smaller cell volume and improved geometry, but also to a shorter combustion tube. The combination of these modifications have provided for a significant decrease in detector dead volume.

Electrolytic conductivity detectors have been utilized for the determination of a variety of chlorine-, nitrogen-, and sulfur-containing compounds in different sample media[5–9]. The Hall detector is known to exhibit a rapid decrease in response following the injection of lipid extracts that have not been subjected to rigorous clean-up[10].

However, a systematic study of the performance of the Hall detector under severe injection loading of biological sample extracts has not been reported. Another disadvantage of this detector is the incompatibility of gas chromatographic (GC) liquid phases containing halogenated compounds. Bleed from columns such as OV-210 or QF-1 produce inordinately high noise levels. For this reason a surface-bonded Carbowax 20M column was evaluated for use with the detector. We were also interested in determining how this extremely low loaded column would perform under repetitive injections of cleaned-up lipid extracts.

Aue *et al.*[11,12], developed solid supports with surface-bonded Carbowax 20M and reported on their application in GC. These investigators coated Carbowax 20M on an acid-washed support, and then after heat conditioning, removed the non-bonded liquid phase by solvent extraction. A thin layer of the liquid phase remained bonded to the support surface and functioned chromatographically. The employment of this unusual column packing for the determination of organochlorine pesticides in biological extracts has not been extensively investigated. Winterlin and Moseman[13], however, prepared and used surface-bonded Carbowax 20M columns for GC of a large number of pesticides and metabolites. In this article, we will report on the application of the Carbowax 20M column and the Hall detector for the determination of chlorinated pesticides in human adipose-tissue and human milk samples at sub-ppm concentration levels.

Gel-permeation chromatography (GPC) was utilized to remove additional lipid material from certain extract samples and proved to be valuable as an adjunct clean-up technique[14–16].

EXPERIMENTAL

Reagents

Analytical reference standards (oxychlordane, *trans*-nonachlor, dieldrin, β-HCH, heptachlor epoxide, *p,p'*-DDE and *p,p'*-DDT) were >99% purity.

Toluene, ethyl acetate and methanol were pesticide quality or equivalent.

Apparatus

A Tracor MT 222 gas chromatograph equipped with a Tracor 700 Hall electrolytic conductivity detector and a pulsed linearized ^{63}Ni electron-capture detector was used. The GC columns were 1.8 m × 4 mm I.D. borosilicate glass, packed with 1.5% OV-17–1.95% QF-1 on Gas-Chrom Q (80–100 mesh), 5% OV-1 on Chromosorb W (80–100 mesh), and surface-bonded Carbowax 20M on acid-washed Chromosorb W (80–100 mesh). A 2-cm layer of 5% Carbowax 20M on Chromosorb W was placed on the injection side of the Carbowax column to help protect the column from build-up of lipid material. The Carbowax column was operated at 175 or 185° with a helium flow-rate of 50 ml/min; the OV-17–QF-1 and OV-1 columns were operated at 200° with a 5% methane–95% argon flow-rate of 60 ml/min. Other parameters for the Hall detector were: 18.5 cm × 2 mm I.D. quartz combustion tube; furnace temperature 820°; hydrogen flow-rate, 20–40 ml/min; transfer line, 270°; methanol flow-rate through detector cell, 0.3–0.5 ml/min. Other parameters for the electron-capture detector were: detector, 275°; transfer line, 230°.

An Autoprep Model 1001 gel-permation chromatograph (Analytical Bio-

chemistry Labs., Columbia, Mo., U.S.A.) equipped with a 350 × 25 mm I.D. glass column containing 60 g of 200–400 mesh Bio-Beads SX3 (Bio-Rad Labs., Richmond, Calif., U.S.A.) was used for additional clean-up of certain sample extracts.

A Go-Getter gas purifier was used for helium (General Electric, Schenectady, N.Y., U.S.A.). It is distributed by Alltech (Arlington Heights, Ill., U.S.A.).

Method

Human adipose-tissue and human milk samples were extracted and cleaned up using a modification of the Mills, Olney and Gaither procedure (MOG)[17].

These extracts were then composited for confirmatory analyses. Additional clean-up on the MOG 15% fractions from the adipose-tissue extracts and MOG 6% fractions from the human milk extracts was accomplished with GPC using Bio-Beads SX-3 and a toluene–ethyl acetate (1:3) elution solvent. The elution rate was approximately 5 ml/min (ref. 15). The first 100 ml of solvent, containing lipids, were discarded. The next 95 ml, containing the pesticides, were collected.

The 6% fractions from the adipose-tissue extracts were analyzed for oxychlordane, *trans*-nonachlor, β-HCH, *p,p'*-DDE and *p,p'*-DDT. The 15% fractions were analyzed for dieldrin. The 6% fractions from the milk extracts were analyzed for *p,p'*-DDE. Each sample was analyzed by both the Hall detector and the electron-capture detector. The Carbowax 20M column was used for determination of oxychlordane, *trans*-nonachlor, *p,p'*-DDE, *p,p'*-DDT, and dieldrin on the Hall detector. An OV-1 column was used for determination of β-HCH because of an interference from heptachlor epoxide on the Carbowax 20M column. An OV-17–QF-1 column was used for the determinations by electron-capture GC.

RESULTS AND DISCUSSION

The MOG 6% fractions from human adipose-tissue were analyzed by both electron capture and electrolytic conductivity GC. To compare graphically the values obtained by the two detector systems, regression lines were calculated by the least squares method and appear in Fig. 1 (a–e). As indicated, the data conform to a straight line ($y = a + bx$). The coefficients of correlation (0.895 to 0.984) indicate a high degree of association between the results obtained by the detectors. The standard error of estimate for each line is denoted by S_y. The $\pm 2S_y$ values plotted on each figure represent the boundary area which enclosed 95% of the data pairs. In the course of these determinations, innumerable injections (12 mg tissue equivalent per injection) were made without observing any deterioration in the Hall detector sensitivity or column performance. The glass demister tubes in the injection ports of the gas chromatograph were changed daily.

It was recognized that a potential existed for decreased response of the electrolytic conductivity detector if the 15% MOG fractions were analyzed on a routine basis without additional clean-up. To investigate this aspect of the study, a number of injections were made of a 15% fraction obtained from a human adipose-tissue extract. Fig. 2 illustrates the variations in response resulting from these injections. By plotting the response elicited from an oxychlordane standard after several sample injections, an indication of analytical performance was obtained. Sensitivity was highly dependent upon the accumulation of lipid residue in the demister tube in the injection

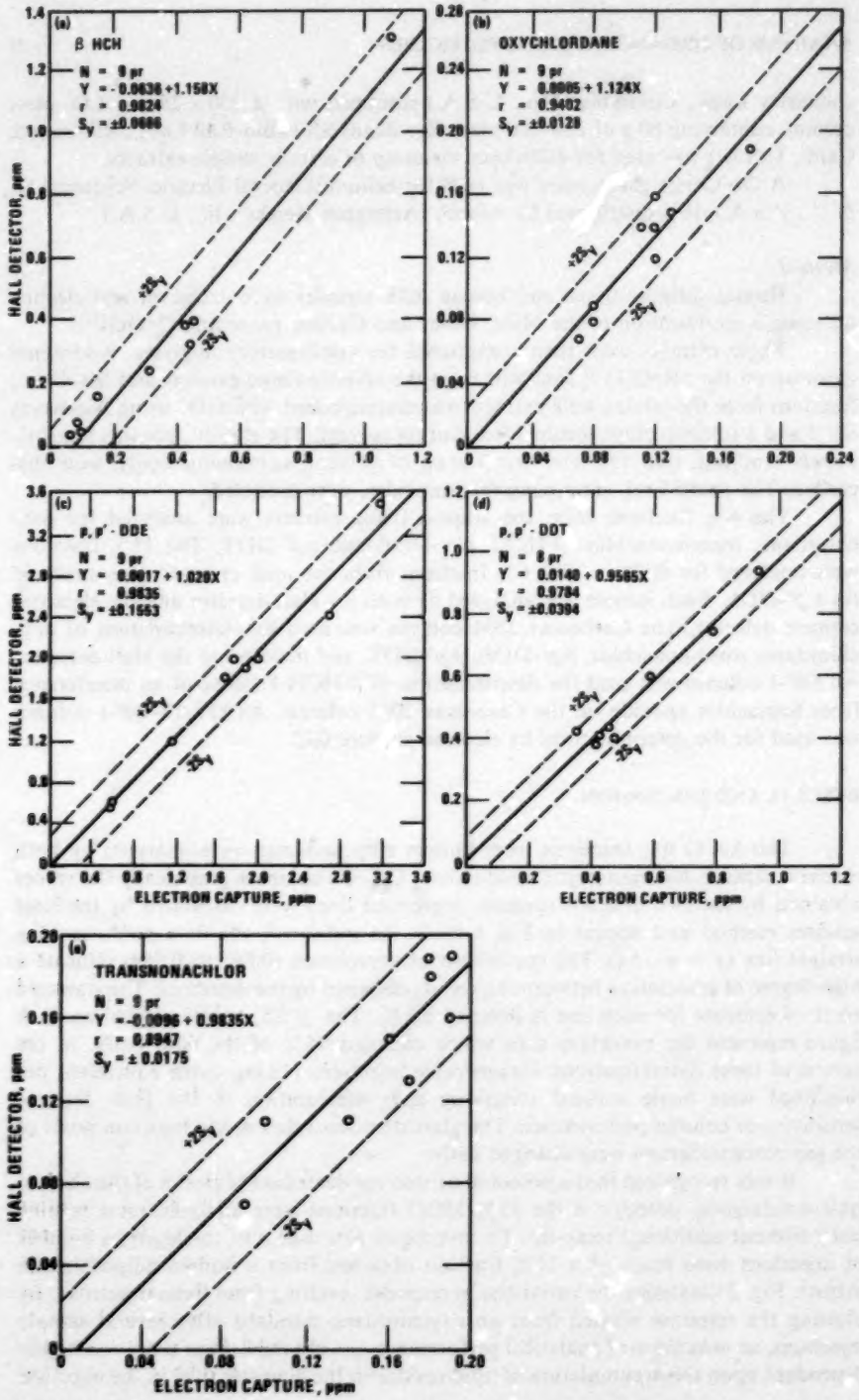

Fig. 1. Linear regression curves of analytical results on human adipose-tissue samples using the electron-capture and Hall detectors.

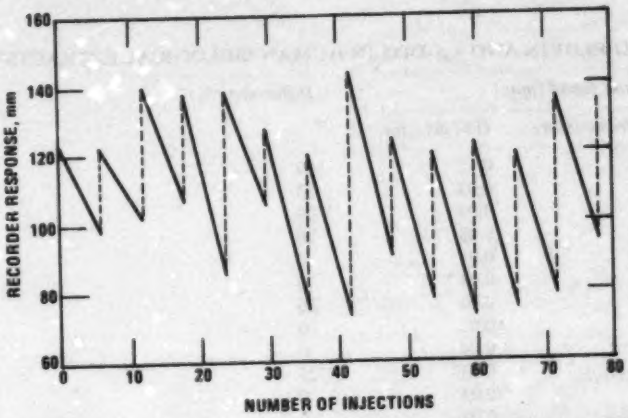

Fig. 2. Variation of response of the Hall detector to oxychlordane (600 pg per injection) after repeated injections from the 15% fraction from human adipose-tissue extracts without additional clean-up with GPC (22 mg tissue equivalent per injection).

port. After steadily decreasing response resulting from six sample injections (indicated by a solid line), the demister tube was replaced with a clean one. After overnight equilibration the response returned to approximately previous levels (indicated by a broken line). Lipid material collected in the demister trap also contributed to decreased column resolution and peak distortion since these symptoms disappeared after a clean demister tube was installed. An occasional reconditioning of the column at 230–240° for overnight periods was also beneficial in restoring column performance.

Since the Carbowax 20M column was new, the sharp increase in response after approximately ten injections of samples containing lipids was not surprising. This probably represents a phenomenon which has been commonly observed before when injections of lipid-containing extracts on new GC columns cover active sites and result in better column performance after sustained use[18].

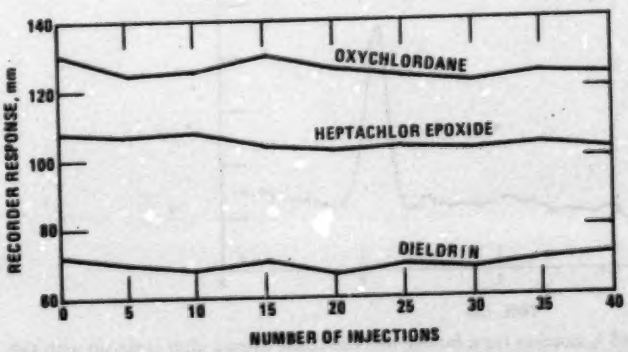

Fig. 3. Variation of response of the Hall detector to pesticide standards (600 pg per injection) after repeated injections from the 15% fraction of human adipose-tissue extracts after GCP clean-up (22 mg tissue equivalent per injection).

TABLE I

DETERMINATION OF DIELDRIN AND *p,p'*-DDE IN HUMAN BIOLOGICAL EXTRACTS*

Sample	*Amount found (ppm)*		*Difference (%)* **
	Electron capture	*Hall detector*	
Adipose tissue	0.11	0.12	9
Adipose tissue	0.02	0.03	50
Adipose tissue	0.07	0.09	29
Adipose tissue	0.10	0.08	20
Milk	0.01	0.01	0
Milk	0.04	0.04	0
Milk	0.05	0.04	20
Milk	0.02	0.02	0
Milk	0.04	0.04	0
Milk	0.04	0.03	25
Milk	0.03	0.03	0
Milk	0.08	0.08	0
Milk	0.03	0.04	33

* Adipose tissue was analyzed for dieldrin; milk was analyzed for *p,p'*-DDE.

** Calculated using the electron-capture result as the accepted reference value. Average difference, 14%.

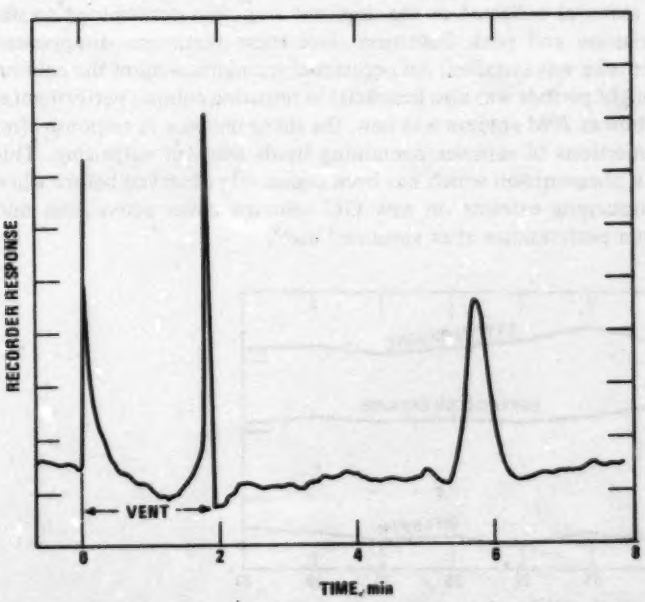

Fig. 4. Chromatogram of 15% fraction from human adipose-tissue extract after clean-up with GPC (30 ppb (10^9) dieldrin). Injection: 5 µl/1.0 ml (13 mg tissue equivalent). Detector: Hall electrolytic conductivity; column: Carbowax 20M; oven temperature: 175°; carrier gas flow-rate: 50 ml/min; reaction gas flow-rate: 20 ml/min; furnace temperature: 820°.

After 72 injections from the 15% fraction, the sensitivity declined to a low level and did not increase by simply replacing the demister trap. At this point, certain maintenance procedures for the Hall detector were performed. A new combustion tube and ion-exchange resin were installed and the conductivity cell was cleaned. The 2-cm layer of 5% Carbowax 20M and the glass-wool plug at the injection end of the column was replaced. The column was heated at 230–240° overnight. The combustion tube showed definite signs of use by its opaque appearance. These procedures produced a significant increase in sensitivity and column resolution and performance returned to previous levels.

Fig. 3 indicates the variation of response of pesticide standards during a series

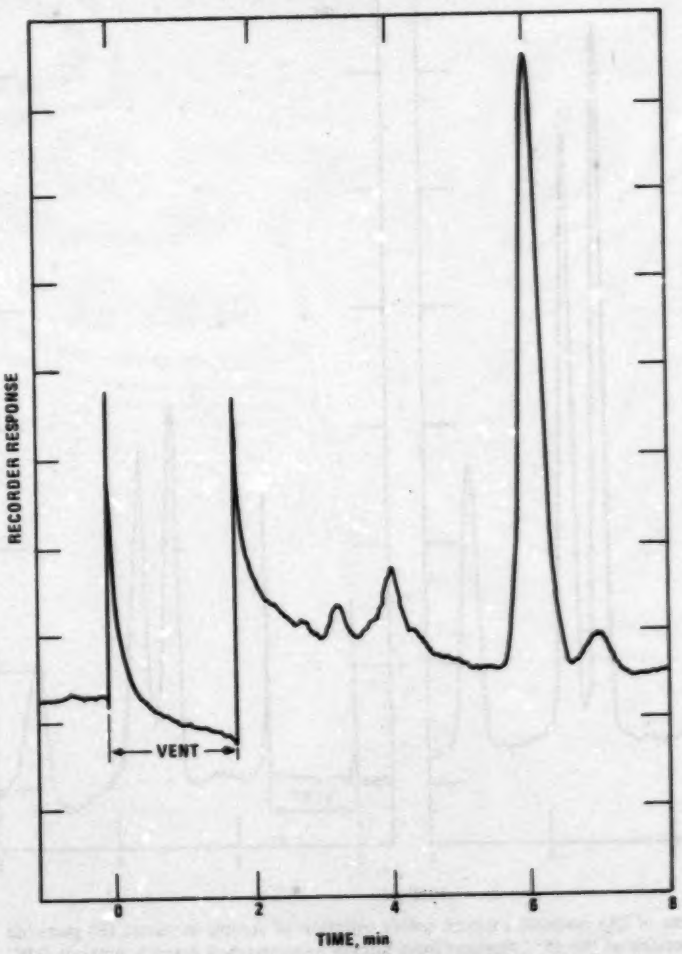

Fig. 5. Chromatogram of 6% fraction from human milk extract after clean-up with GPC (44 ppb *p,p'*-DDE). Injection: 3 μl/ ml (42 mg tissue equivalent); detector: Hall electrolytic conductivity; oven temperature: 185°; for other instrument conditions, see Fig. 4.

of injections of a MOG 15% fraction which had been additionally cleaned up with GPC. As many as twenty injections could be made during a day without changing the demister trap. After 40 injections no significant decrease in response or column resolution was observed.

The comparison of Figs. 2 and 3 clearly demonstrates the usefulness of GPC in the pesticide laboratory. By removing excess lipids, instrument "down time" is reduced by decreasing contamination of the analytical system. Also much lower levels

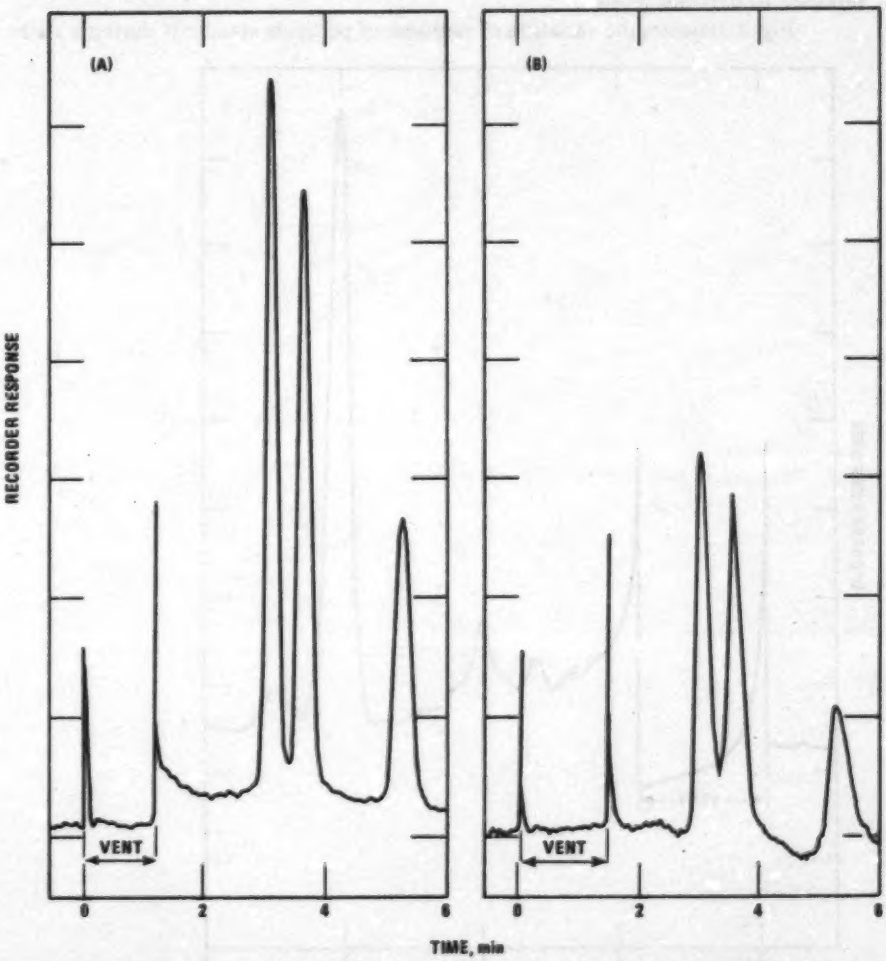

Fig. 6. Chromatograms of (A) pesticide mixture before injection of sample extracts; (B) pesticide mixture after six injections of the 15% fraction from human adipose-tissue extracts without GPC clean-up (22 mg tissue equivalent/injection). Pesticide mixture = 600 pg oxychlordane, heptachlor epoxide and dieldrin in order of elution. Detector: Hall electrolytic conductivity; oven temperature: 185°; for other instrument conditions, see Fig. 4.

of pesticide residues can be detected and quantitated. Under conditions of heavy use the combustion tube, ion-exchange resin, electrolytic cell and column will undoubtedly require service more often if GPC or some other additional clean-up is not used on extracts of high lipid content. Fig. 2 illustrates the surprising ability of the low-load Carbowax 20M column to withstand repeated injections of high lipid content and continue to perform well.

Dieldrin and *p,p'*-DDE were quantitated in the MOG 15% and [illegible]% fractions from human adipose-tissue extracts and human milk extracts respectively. The results appear in Table I. The relatively close agreement of the data enhances the value of the electrolytic conductivity detector as an analytical tool. Figs. 4 and 5 illustrate representative gas chromatograms obtained from these analyses. The chromatograms indicate the feasibility of using the Hall detector for determination of chlorinated pesticides in biological samples, even at concentration levels well below 1 ppm. Because of the amount of lipid material occurring in the fractions, additional clean-up of extracts was achieved with GPC. This allowed a number of injections over the course of a day without a decrease in performance of the Hall detector or column. GPC clean-up also aided in preventing "down time" because of the need to change a demister tube after only a few injections.

Fig. 6 shows a comparison of the chromatograms depicting a mixture of pesticide standards before and after repetitive injections of six sample extracts of the 15% fraction from a human adipose-tissue extract. A sharp decline in response and resolution of the compounds is clearly evident. This was not observed when the 15% fractions were subjected to further clean-up by GPC.

CONCLUSION

The Hall detector and Carbowax 20M column have been shown to be valuable for confirmation and determination of chlorinated pesticides in biological samples. Determinations at concentrations down to 0.01 ppm are obtainable at optimum levels of instrument performance. GPC on samples of high lipid content is recommended to prevent contaminants in the system which would necessitate more frequent maintenance of the electrolytic conductivity system.

Successful application of the Hall detector to confirmation of organochlorine pesticides in biological and environmental samples would for many laboratories provide an inexpensive substitute for combined GC–mass spectrometry (MS) in many instances. For those laboratories possessing GC–MS capabilities, it would free the instrument for more difficult identification work.

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